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CITRUS RESEARCH CONFERENCE

1959

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U.S. DEPARTMENT

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✓✓ UNITED STATES DEPARTMENT OF AGRICULTURE
✓ Agricultural Research Service
Western Utilization Research and Development Division

✕ PROGRAM AND ABSTRACTS OF PAPERS
CITRUS RESEARCH CONFERENCE

January 27, 1959~

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California

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FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in California and Arizona the latest results of research on the chemistry and technology of citrus fruits and fruit products carried on by laboratories of the Agricultural Research Service, U. S. Department of Agriculture. The following Divisions are participating in this year's Conference:

Western Utilization Research and Development Division

Western Regional Research Laboratory (Division headquarters), Albany, California

Fruit and Vegetable Chemistry Laboratory, 263 South Chester Ave., Pasadena, Calif.

Southern Utilization Research and Development Division

U.S. Citrus Products Station, Winter Haven, Florida

U.S. Fruit and Vegetable Products Laboratory, Weslaco, Texas

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C I T R U S R E S E A R C H C O N F E R E N C E

Tuesday, January 27, 1959

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Pasadena, California--Presiding.

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RECENT RESULTS ON THE CHEMISTRY OF CITRUS FLAVONOIDS

R. M. Horowitz and Bruno Gentili

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Work on the isolation and proof of structure of citrus flavonoids has continued during the past year. The objectives of this work have been to clarify the relationships between type of flavonoid and darkening and bitterness of lemon products, as well as to provide pure compounds of known structure for metabolic studies.

Up to the present time the following flavonoids have been isolated from extracts of lemon peel: hesperidin, diosmin, eriodictyol, eriodictyol glycoside, luteolin, chrysoeriol, isorhamnetin, quercetin, apigenin, limocitrin A, limocitrin A glycoside, limocitrin B, and limocitrin B glycoside. The structure of limocitrin A, a new flavonoid, was reported earlier as 3, 5, 7, 4'-tetrahydroxy-8, 3'-dimethoxyflavone. This has been confirmed by converting limocitrin A to its 5-methyl ether and showing that this derivative is identical with synthetic 3, 7, 4'-trihydroxy-5, 8, 3'-trimethoxyflavone. Limocitrin B, also a new flavonoid, has been shown to be the 6-methoxy derivative of limocitrin A. This follows from degradation experiments and from application of the spectroscopic procedures, discussed here last year, for the determination of the structure of flavonols. The probable structures of the crystalline glycosides of limocitrin A and B will be reported. A number of related phenolic compounds have been isolated from lemon peel including scopoletin, umbelliferone, sinapic acid, and p-coumaric acid.

The relationship between bitterness and the chemical structure of flavonoid glycosides is being studied. Naringin, which has long been recognized as the chief bitter constituent of grapefruit, was thought to be the 7-rutinoside of the aglycone naringenin. Neohesperidin, one of the principal flavonoids of the bitter orange, is isomeric with hesperidin (hesperetin 7-rutinoside) and differs from it in the nature of the sugar residues. We have found that both naringin and neohesperidin undergo cleavage in alkali to yield, in each case, the same phloroacetophenone rhamnoglucoside.

It is of considerable interest that both



neohesperidin and the phloracetophenone rhamnoglucoside are bitter, though not to the same degree as naringin. The significance of these findings is thought to be as follows: (1) Naringin is not a rutinoside and this probably

accounts for the puzzling fact that its solubility and taste differ very markedly from those of hesperidin. (2) The disaccharide of both naringin and neohesperidin is identical. It is a rhamnoglucoside (called "neohesperidose"), and is different from but isomeric with rutinose, the only other known rhamnoglucoside. The structure of this disaccharide is unknown, but it is currently being worked on. (3) The presence of "neohesperidose" in flavonoid glycosides is probably intimately associated with the property of bitterness. At least one other bitter citrus flavonoid ("poncirin") has been discovered which appears to contain this disaccharide.

† PHARMACOLOGICAL STUDIES ON LEMON OIL CONSTITUENTS †

A. N. (Booth, M. S. (Masri, D. J. (Robbins, O. H. (Emerson,
F. T. Jones, and F. (DeEds

Western Utilization Research and Development Division
Pharmacology Laboratory, Albany, Calif.

The isolation and identification of 8 compounds from lemon oil was reported by Stanley and Vannier at the Citrus Conference last year. These compounds have now been evaluated by the Pharmacology Laboratory for their physiological effects in animals.

Since lemon oil is used as a food additive, the question is raised as to whether any of its constituents are absorbed from the gastrointestinal tract after oral ingestion and if so what metabolic changes, if any, take place in the animal body. The metabolic fate of coumarin was investigated first, because many of the components in lemon oil are derivatives of coumarin. Fortunately, the structure of coumarin is such that it lends itself to the same methods used in studying the metabolism of the citrus flavonoids.

Through use of the same two-dimensional paper chromatographic technique which was devised for the flavonoid studies, 10 metabolites have been identified in the urine of rats and rabbits after oral ingestion of coumarin. The major metabolite was ortho-hydroxyphenylacetic acid, which is formed from coumarin by loss of one carbon from the lactone ring. The other metabolites identified were trans-ortho-coumaric acid, ortho-hydroxy-phenylpropionic acid, 3-hydroxycoumarin, 4-hydroxycoumarin, 7-hydroxycoumarin, and the glycine conjugates of ortho-coumaric acid and ortho-hydroxyphenylpropionic acid.

Rats were given 5, 7-dimethoxycoumarin by stomach tube. None of the metabolites arising from coumarin were detected. However, unquestionable evidence of absorption from the gastrointestinal tract was obtained, since the compound given was excreted in the urine along with 3 other metabolites which have not as yet been identified.

Evidence of absorption was also obtained when rats were given 5-geranox-7-methoxycoumarin. This was based on the finding in the urine of the compound given plus a metabolite which was chromatographically similar to one of the three metabolites mentioned when 5, 7-dimethoxycoumarin was administered.

Pharmacological studies involving the application of psoralen derivatives isolated from lemon oil to the skin of guinea pigs, followed by exposure to a sunlamp, were performed. These studies were based on reports that certain naturally occurring furocoumarins possess photosensitizing properties. Several years ago it was established that 8-methoxypsoralen was the active substance in plant extracts which had been used by the Egyptians for centuries as pigmenting agents. Since this compound is a photo-sensitizer it has been proposed for use to facilitate tanning of the skin for cosmetic purposes as well as

to increase the tolerance of skin to sunlight. It has now been shown that, when this drug is taken by humans either orally or by topical application (5 to 20 mg. daily) followed by exposure of the skin to ultraviolet light, a thickening of the horny layer of the skin takes place. It is this thickening of the corneum that is largely responsible for the increased resistance of skin to sunburn after psoralen therapy. Apparently the increased tanning effect is achieved primarily by retention of melanin rather than by an enhancement in the formation of new melanin. Unless it can be clearly established that no toxic effects will result from continuous ingestion of 8-methoxypsoralen year after year, the desirability of using such a drug is highly questionable.

The application of 8-methoxypsoralen to the skin of guinea pigs followed by exposure to a sunlamp produced erythema or redness at the site of application. Only 5 micrograms of material were required to elicit a positive effect, whereas untreated animals similarly exposed to the sunlamp showed no reaction. Dosages as high as 500 micrograms of the following compounds isolated from lemon oil were tested on guinea pig skin and found to be negative:

- (1) 5-geranoxypsoralen
- (2) 7-methoxy-5-geranoxypsoralen
- (3) 8-geranoxypsoralen
- (4) 5,7-dimethoxycoumarin

If we assume that the lack of photosensitizing action of these compounds on guinea pig skin would be the same for human skin, then these lemon oil constituents can be regarded as being non-toxic.

7 EFFECT OF FLAVONOIDS ON THE PITUITARY ADRENAL AXIS 4

Floyd (DeEds

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Beneficial effects of flavonoids have been claimed in a wide variety of conditions in studies with experimental animals and in clinical use. Many, but not all, of these conditions have been reported to be characterized by an increased capillary permeability and/or fragility. The antioxidant action toward epinephrine and ascorbic acid and the inhibitory action on hyaluronidase possessed by the flavonoids in varying degrees have been used to explain the alleged ability of flavonoids to correct capillary abnormality. Nevertheless, several puzzling questions remain. Some of the clinical conditions reported to be benefited by flavonoids do not obviously involve capillary abnormalities.

The effects of flavonoids in experimental animals are usually acute phenomena associated with a single administration of a flavonoid. In contrast, beneficial results claimed in clinical trials have not been apparent until flavonoid administration has been continued for a time, often 3 to 6 weeks. Another discrepancy between experimental animal and clinical investigations is found in the dosages employed, 100 mg./kg. or more in the former as compared with a dose of 60 to several hundred mg. per individual given daily in the latter. These differences between experimental animal and clinical investigations point to the need for a new approach to our understanding of mechanism of flavonoid action.

The discovery that oral administration of a number of flavonoids to experimental animals and human volunteers resulted in the urinary excretion of a variety of hydroxyaromatic acids suggested a new approach. Specifically, this discovery suggested that the flavonoids per se or certain of their metabolites might influence the pituitary-adrenal axis in a manner comparable to that reported for salicylic acid and related compounds. A criterion for such an action is the involution (decrease in weight) of the thymus gland in weanling rats. Such involution is known to be produced by cortisone and by ACTH which stimulates production of cortisone by the adrenal glands.

The feeding of quercetin, dihydroquercetin, or eriodictyol to weanling rats produced a significant decrease in thymus gland weights, expressed as percent of total body weights. This involution of the thymus did not occur in animals from which the adrenal or pituitary glands had been removed. The possibility that flavonoids such as quercetin and eriodictyol can directly or indirectly stimulate cortisone production makes it possible for the first time to explain a wide spectrum of seemingly unrelated physiological effects attributed to these compounds.

MATERIALS BALANCES IN THE CONCENTRATION OF ORANGE JUICE

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U.S. Fruit and Vegetable Products Laboratory
Winter Haven, Florida

Differences between total soluble solids in the product of concentrate plants and total soluble solids purchased have given rise to the opinion in industry that considerable losses of fruit solids take place in processing. In preparation for a study of this problem in a commercial plant an investigation of materials balances was undertaken in the laboratory, using equipment in which both the feed juice and product could be accurately weighed. Properties which were measured in both single strength and concentrate juices included titratable acidity, Brix by pycnometer, Brix by refractometer, soluble solids and total solids by vacuum drying. Each of these properties was evaluated as a basis for calculating apparent gain or loss of solids during evaporation.

A series of 11 evaporations was made with juice containing graduated levels of pulp from early-season unfrozen oranges and from lightly damaged Queen oranges and centrifuged mid-season juice from a processing plant. Materials balances calculated from each method of evaluation fell within 2% of product weights, except that refractometer balances varied as much as 4%. This indicates that titratable acidity, pycnometer Brix, total solids or soluble solids by vacuum drying are reasonably reliable bases for calculating materials balances. Refractometer values give only slightly less reliable balances.

Following the laboratory tests, a materials balance study was made on a commercial orange concentrate evaporator. Samples of evaporator feed juice, cutback juice, addback concentrate, and final product were collected continually over a 72-hour cleanup-to-cleanup operation, and brought to the laboratory for analyses. Records were kept of weights or volumes of all materials entering and leaving the various operations. It was necessary to estimate (1) addback losses (1 bbl. in 500), (2) juice remaining in blend tanks at the end of the run, and (3) sampling and broken cans of product (3 cases per shift).

The balance for the evaporating, blending, and filling operation by acid titration showed a solids loss of 1.1%; by refractometer solids a gain of 1.3%; by total solids by drying a loss of 0.5%; and by soluble solids by drying a gain of 0.8%. These results indicate that in-plant losses of fruit solids were not appreciable.

The weight of soluble solids purchased (plant records) was compared with the weight of solids found in the evaporator feed juice less solids from addback plus solids in cutback. All solids were determined as solubles by vacuum drying. The yield of solids in juice from the extractor was 6% less than that indicated by the records on the fruit received.

This work has demonstrated methods that are suitable for use in determining materials balances for concentrate plant operations. In the laboratory and the single plant operation observed there were no serious losses of fruit solids between finishers and final product. These conclusions may or may not apply to other plants.

BIOCHEMISTRY OF CAROTENOID FORMATION IN GRAPEFRUIT

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In attempting to improve the processing quality of Texas red and pink fruit it has been found that there is a need for more knowledge about the chemistry of the carotenoid pigments and the relationship of carotenoid properties to processing characteristics.

The seasonal development and decline of the carotenoids in colored grapefruit has been followed for 4 years. A previous report by Lime *et al.* provides data for the fifth year. In all years for which data are available the maximum lycopene content evidently occurred between August 15 and September 10. The beginning of decline coincides with decreased temperatures but other factors have not been excluded.

An experiment was conducted holding one tree at ambient temperature and cooling another. The results are without statistical significance because of small sample size, but the lycopene in the cooled tree decreased while in the control tree it increased.

Lycopene has been found in Marsh white and Duncan white grapefruit of Texas and Florida. This indicates that the ability to synthesize these pigments existed before mutation to colored varieties.

It is possible that the chemical difference between white and colored grapefruit is in the leaves and stems so that the colored fruit is provided with different nutrition leading to an accumulation of pigments; or it may be that the fruit received identical nutrition and a chemical difference in the fruit caused an accumulation of pigments. Red fruit was grafted on trees of the white variety and white fruit on trees of the red variety. The characteristics of the fruit were unchanged.

The distribution of lycopene in developing red fruit is not uniform. The distribution is similar to the distribution of dye imbibed through the stem of the fruit, as though a precursor enters the fruit through the stem and is converted into lycopene by the tissues it passes through.

Biochemical studies involving the use of radioactive tracers have shown that mevalonic acid is a precursor for the carotenoids. Labeled mevalonic acid injected into tomatoes is incorporated into the carotenes 10 to 50 times as efficiently as labeled acetic acid. Evidence has been gathered to indicate that mevalonic acid is incorporated into the carotenes through the formation of a compound which is apparently a hydrocarbon with a pair of conjugated double bonds and several isolated double bonds.

IDENTIFICATION OF THE CARBONYL COMPOUNDS OF LEMON OIL

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In view of the fact that new and powerful analytical tools have recently become available, and that the latest definitive work in the field of chemistry of citrus oils was carried out with older and less sensitive techniques, a re-investigation has been made of the isolation and identification of the carbonyl compounds of lemon oil. In the earlier work only citral had been positively identified in California lemon oil, although the presence of several others was indicated. Interest in the chemistry of the carbonyls in lemon oil is based on their importance as natural flavoring components and as possible end products resulting from the oxidation of other compounds.

Because of the complexity of lemon oil it was necessary to use a number of techniques for the separation and identification of the components. These included fractional distillation, column chromatography, and gas partition chromatography. Using these techniques octanal, nonanal, decanal, and citral were isolated from lemon oil and positively identified. Tentative identifications were made for undecanal, dodecanal, and citronellal. Several other carbonyls were shown to be present in trace quantities but they have not been identified.

The presence of two forms of citral in lemon oil was confirmed by the separation of their 2,4-dinitrophenylhydrazones on column chromatography. The relative amounts of each form was determined by a spectral method. An analysis was made on the two forms of citral in lemon grass oil to determine whether it would be possible to detect addition of lemon grass citral to lemon oil. No differences were found in the ratio of the two forms of citral from lemon grass oil and lemon oil.

Some foreseeable applications of these investigations are: to aid in developing objective methods to measure the quality of lemon oil, development of methods to detect addition of various substances to authentic oils, and to improve oil processing techniques so as to maintain the maximum amount of desirable constituents and remove the undesirable constituents.

APPLICATION OF THE VANILLIN-PIPERIDINE METHOD
TO THE ANALYSIS OF CITRUS OILS

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The specific colorimetric vanillin-piperidine method of analysis for citral first reported at the 1958 Conference has been used in further studies of the effect of water to fruit ratios on the citral content of lemon oils during their extraction from the peel. Wide variations in ratios caused little if any change in the citral content of the processed oils. This confirms similar results obtained in the laboratory with model systems.

Data are presented on the citral content of a number of lemon oils produced under different cultural and processing conditions. Some of the samples examined were also tested by an independent laboratory with a newly developed barbituric acid condensation method. The results obtained with the two methods showed good agreement.

In addition to its use for analysis of citral the vanillin-piperidine reagent was found to be suitable for the quantitative determination of the non-terpenic alpha-beta unsaturated aldehydes in lemon oil. The red color which develops when the reagent is allowed to react with the 2-monoenals and the 2,4-dienals has an absorption maximum at 587 m μ . Crotonaldehyde was too unstable to permit the preparation of a straight line standard curve, whereas a straight line curve was obtained with 2-hexenal. The time required for maximum development of color for the monoenals was 45 minutes at room temperature, while it was 2-3/4 hours for 2,4-dodecadienal.

Application of the vanillin-piperidine method of analysis to the unsaturated aldehydes in citrus oils, although not providing as specific data as obtained for citral, should prove useful in studying the composition of flavoring components of citrus oils in which the simple aliphatic enals are important.

APPLICATION OF CHROMATOGRAPHIC TECHNIQUES TO THE ANALYSIS OF CITRUS OILS

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Work on the chemistry of the coumarins in lemon oil has been continued to complete the identity of those compounds which have proved useful in developing new methods of analysis for establishing the purity of lemon oils. Structures of two of these compounds have been confirmed by synthesis, i. e., 5-isoamyleneoxypsoralen (isoimperatorin) and 5-isoamyleneoxy-7-methoxycoumarin. This brings the number of coumarins isolated and identified from lemon oil to 7 out of a total number of 10.

The methods of analysis previously described for detecting addition of other citrus oils to lemon oil have been adapted for detecting addition of the so-called "sun esters" (methyl salicylate and beta-homomenthyl salicylate) as adulterants. These compounds have been added to lemon oils to enhance their characteristic UV spectral absorption at 315 mμ. Previously no method has been available for detecting these compounds in citrus oils.

A method has been devised for removing the mixture of carbonyl compounds from citrus oils and analyzing the mixture by means of gas partition chromatography. The carbonyls are converted to water-soluble betaine hydrazones which on removal of the water-insoluble materials (esters, alcohols, and hydrocarbons) are treated with formaldehyde to regenerate the mixture to the original carbonyls. Mildness of the reaction makes it particularly suitable for analysis of the sensitive flavoring components of citrus oils. Conditions of the regeneration step are critical for complete conversion. Of the carbonyl components in lemon oil normal octanal is most rapidly regenerated, whereas citral is only slowly regenerated. By running two regeneration steps, one at 3 °C. for 4 hours and the other at room temperature for 24 hours, it is possible to differentiate between certain of the saturated and unsaturated carbonyls.

Use of this new technique will enable one to obtain for the first time a complete picture of the carbonyl compounds present in essential oils. Its use will offer a convenient approach to an evaluation of the contribution these particular compounds make to the characteristic flavor and aroma of essential oils. A further useful application of the method will be to study the products of oxidation of citrus oils resulting from storage and mishandling.

LIST OF CITRUS PUBLICATIONS
January 1, 1958, to December 31, 1958

Western Utilization Research and Development Division
Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California

EFFECT OF VARIED STORAGE TEMPERATURES ON MICROORGANISMS
IN FROZEN CONCENTRATED ORANGE JUICE.

E. R. Wolford.

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S. H. Vannier and W. L. Stanley.

Jour. Assoc. Offic. Agr. Chem. 41(2):432-435, May, 1958.

A REVIEW OF THE CHEMISTRY OF CITRUS FLAVORS.

W. L. Stanley.

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W. L. Stanley.

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EXAMINATION OF LEMON OIL BY GAS-PARTITION CHROMATOGRAPHY.

R. A. Bernhard.

Food Res. 23(2):213-216, March-April, 1958.

OCCURRENCE OF COUMARIN ANALOGUES IN LEMON JUICE.

R. A. Bernhard.

Nature 182, page 1171, 1958.

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W. L. Stanley, R. C. Lindwall, and S. H. Vannier.

Jour. Agr. and Food Chem. 6(11):858-860, Nov., 1958.

PROCESS FOR PRODUCTION OF ERIODICTYOL.

R. M. Horowitz.

U.S. Patent 2,857,318, October 21, 1958.

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SALTS.

R. J. McColloch and B. Gentili.

U.S. Patent 2,845,355, July 29, 1958.

Western Utilization Research and Development Division
800 Buchanan Street, Albany 10, California

METABOLIC FATE OF HESPERIDIN, ERIODICTYOL, HOMOERIDICTYOL,
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THE TOXICITY AND METABOLISM OF DIHYDROQUERCETIN.

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Jour. Amer. Pharm. Assoc., Sci. Ed., 47(3):183-184, March, 1958.

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A. N. Booth, F. T. Jones, and F. DeEds.
Jour. Biol. Chem. 233(2):280-282, Aug., 1958.

EFFECT OF CERTAIN FLAVONOIDS ON PITUITARY-ADRENAL AXIS.

M. S. Masri and F. DeEds.
Proc. Soc. Expt. Biol. and Med., Dec., 1958.

Southern Utilization Research and Development Division
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509 West 4th Street, Weslaco, Texas

PRODUCTION OF CANNED PULP-FORTIFIED RED GRAPEFRUIT JUICE.

B. J. Lime, T. S. Stephens, and F. P. Griffiths.
Agricultural Research Service Circular, ARS-72-12, 10 pp., Jan., 1958.

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T. S. Stephens.
Citrus Indus. 39(1):16-19, 1958.

PARTITION SEPARATION OF CAROTENOIDS BY SILICA-METHANOL COLUMNS.

A. E. Purcell.
Analyt. Chem. 30(6):1049-1051, 1958.

